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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF NITROPHENOLS WITH A SWEPT-POTENTIAL ELECTROCHEMICAL DETECTOR

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SUMMARY

The advantages of a swept-potential electrochemical detector are demonstrated by application to the high-performance liquid chromatography (h.p.l.c.) of nitrophenols. The primary advantage of the detector is that an additional dimension of information is obtained which can be used to help identify a chromatographic peak and help to resolve overlapping peaks. The detector retains the selectivity of a differential-pulse detector and yet is general enough to minimize the time for development of new procedures and to detect true unknowns. The h.p.l.c. of nitrophenols was studied on C-18 reverse-phase columns, with methanol-acetate buffer mobile phases. Selectivity factors of the mononitrophenols are independent of the composition of the mobile phase from pH 3.25 to 6.00 and from 40 to 55% methanol. The selectivity factors of dinitrophenols are highly dependent on pH. Limits of detection depend on many parameters but are approximately 1 ng injected for the nitrophenols.

This paper is the first in a series to discuss the design and applications of a swept-potential electrochemical detector for high-performance liquid chromatography (h.p.l.c.). The relative advantages of the detector will be illustrated by application to nitrophenols. Future papers will discuss the detailed design and performance of the instrument as well as applications to other types of compounds.

The basic concept of a swept-potential detector using square-wave voltammetry have been discussed by Samuelsson et al. [1] and reviewed by Bratin and Kissinger [2]. Different approaches to swept-potential detectors were reported by Wang et al. [3], Stastny et al. [4], Last [5], and Caudill et al. [6]. The primary advantage of the swept-potential detector is that an additional dimension of information is obtained. The current response as a function of potential can be used to help identify a component and to help resolve two components not completely separated on the column. In addition, the potential for maximum current response is crossed for all components within the potential range of the sweep, thus decreasing the time required to optimize a procedure and allowing the determination of multiple components in one chromatogram, each at its optimum potential. If, as in the

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present case, the data are stored in a computer file, chromatograms can be displayed for any desired potential within the sweep range, or chromatograms for the summations between desired potentials can be displayed. Thus, this detector retains the selectivity of a differential-pulse detector and at the same time is general enough to evaluate true unknowns.

Nitrophenols were selected as the first class of compounds for application of the swept-potential detector for several reasons. First, several nitrophenols are on the list of priority pollutants of the United States Environmental Protection Agency. Second, while the performance of the electrochemical detector was being improved and evaluated, it was desirable to work with a class of compounds which can be detected with a u.v. detector. Third, the literature on electrochemistry of nitrophenols is extensive. Finally, nitrophenols are detected by electrochemical reduction reactions while the majority of previous work with electrochemical detectors has been with oxidation reactions. Applications to reduction reactions are generally considered to be more difficult than to oxidation reactions because of higher background currents from the reduction of hydrogen ions and oxygen. Thus, successful application to reductions would serve as a rigorous test of the detector system.

EXPERIMENTAL

Apparatus

The chromatographic instrumentation was locally assembled from components and designed for isocratic elution. Initially the pump on the instrument was a Haskel pneumatic amplifier pump (No. 26740) as modified by DuPont for their Model 830 chromatograph. Later determinations were made with a Spectra-Physics 8770 pump, which was found to be sufficiently pulse-free for the electrochemical detector. Columns (4.6 × 250 mm) were locally packed with RSIL C18 HL (10 μm) for reverse-phase separations and were preceded by a guard column (4.6 × 50 mm) containing pellicular C-18 resin (Alltech 9260). Valco injectors (either 6-port or 10-port), driven by air actuators, were fitted with 10-μl sample loops. The system was all stainless steel from the solvent reservoir to the detector inlet with 1/8-in. tubing (2.16-mm i.d.) connecting the solvent reservoir to the pump and 1/16-in. tubing (0.25-mm i.d.) was used in the rest of the system. The column effluent was attached to a u.v. detector (Chromatronix model 220), for monitoring at 254 nm, or to the electrochemical detector.

The electrochemical detector was designed and assembled in this laboratory. It consists of a computer-controlled potentiostat and a modified PARC 310 electrode stand and flow cell. The computer system consists of a Z80A processor with 64 kbyte of RAM and dual 5.25-in. floppy-disk drives. The graphics terminal also includes a Z80A and 64-kbyte RAM. The graphics are bit-mapped with 640 points horizontally and 480 points vertically Any alphanumerics or graphics can be dumped to the IDS Prism 80 dot-matrix

printer. The instrument-control and data-acquisition programs are written in assembly language while the data-processing programs are written in BASIC or FORTRAN IV.

All parameters for the detector are selected from a choice displayed on the CRT and are down-loaded from the computer to the potentiostat in digital format. The potentiostat contains a current-interrupt circuit, by use of which the IR drop of the cell can be measured, and a positive-feedback circuit for IR compensation. A direct current offset can be adjusted automatically to give a net current of zero at the start of the voltammetric sweep. These two features are important in a practical instrument and are lacking in the swept-potential detectors previously described [1, 3—6]. During an experiment, a 3-D plot of the data is displayed, as it is acquired, on the upper two-thirds of the CRT while the selected parameters are displayed on the lower third of the CRT. After the experiment, any desired voltammogram or chromatogram can be extracted from the data file, displayed on the CRT, and hard copies made on the printer. Details of the instrument will be provided to interested readers.

Reagents

The nitrophenols used were commercial products that were not purified further. The dinitrophenols contained 15—50% "moisture" as indicated on their labels. Samples were prepared on a weight basis in methanol with concentrations calculated on the basis of an estimated dry weight. Acetate buffers of the desired pH values were prepared by mixing sodium acetate and anhydrous acetic acid with water so that the final concentration of acetate was 0.20 molar. These buffers were filtered through a 0.45-μm nylon filter under vacuum. Mobile phases were made by mixing the acetate buffers with methanol on a volume basis. The methanol (Burdick and Jackson, h.p.l.c. grade) was used unfiltered.

Procedures

Each of the nitrophenols was chromatographed, individually and in mixtures, with a number of mobile phases which differed in the pH of the acetate buffers and in percentage of methanol. Plots of retention time vs. pH or percent methanol were constructed for each compound. Square-wave voltammetry at various values of pH and percent methanol was applied to each nitrophenol, and the limits of favorable electrochemical response were thereby determined.

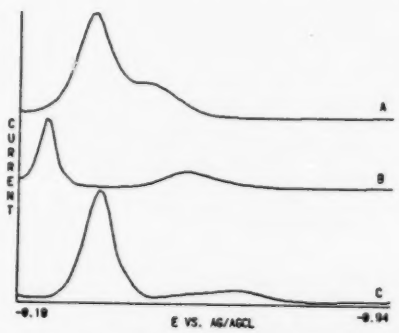
For use with the u.v. detector, the mobile phase was equilibrated with air. When the electrochemical detector was used, the mobile phase was purged vigorously with helium for at least 30 min before the pump was started, and purging was continued for a further 30 min with mobile phase flowing through the column. Oxygen was not removed from the injected samples because the system clearly separated oxygen from the nitrophenols. The outer cell of the PARC 310 was filled with mobile phase and purged with nitrogen.

Although the u.v. cell could have been placed in the effluent stream before the electrochemical cell, this arrangement was not done because of band broadening in the u.v. cell. Also, the u.v. cell allowed enough oxygen to enter the effluent stream that the background currents in the electrochemical cell were excessive. The reverse sequence of the cells is not possible.

At the start of each day, the capillary was removed from the electrode stand, flushed with water and methanol, and then air-dried by use of a vacuum pump. After re-installation, a vacuum pump was attached to the capillary tip to remove any air entrapped in the valve system. Large mercury drops were dispensed continuously as the vacuum pump was disconnected from the capillary tip. If excessive capillary noise was noted, the capillary was replaced with a new or resiliconized capillary. The saturated KCl solution in the Ag/AgCl reference electrode was also replaced daily.

RESULTS AND DISCUSSION

Aromatic nitro compounds are excellent electron acceptors. The electrode reactions are irreversible, however, and result in broad voltammetric peaks. Mononitrophenols give a single peak, whereas the simple dinitrophenols produce two peaks, the first being generally sharper and higher than the second. Figure 1 depicts typical voltammograms and shows that the second peak for 2,6-dinitrophenol is only a shoulder on the first peak. Table 1 summarizes experimental peak potentials for the compounds studied here. Peak potentials are functions of pH and percent methanol, but response is



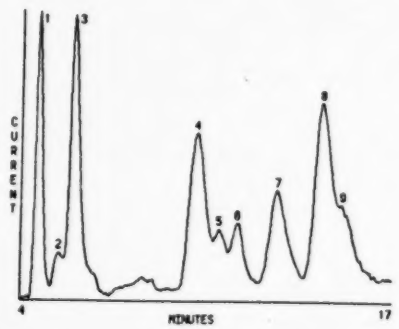


Fig. 1. Square-wave voltammograms: (A) 2,6-dinitrophenol, (B) 2,5-dinitrophenol, (C) 2,4-dinitrophenol. Data taken with 30-Hz square wave in a flow stream as in Table 1.

Fig. 2. Chromatogram of a mixture of nitrophenols. Peaks: (1) 2,6-dinitrophenol (75 ng); (2) unknown impurity; (3) 2,4-dinitrophenol (128 ng); (4) *p*-nitrophenol (150 ng) and 2,3-dinitrophenol (120 ng); (5) *m*-nitrophenol (150 ng); (6) 2,5-dinitrophenol (98 ng); (7) 3,4-dinitrophenol (128 ng); (8) 2,4-dinitro-*o*-cresol; (9) *o*-nitrophenol (150 ng). Currents summed over potential range of −0.06 to −0.61 V vs. Ag/AgCl. Mobile-phase flow rate 1.2 ml min^{-1}. See text for other conditions.

TABLE 1

Voltammetric peak potentials

Compound	Peak potentials[a] (V)		Compound	Peak potentials[a] (V)	
p-nitrophenol	—	−0.58	2,5-Dinitrophenol	−0.15	−0.45
m-nitrophenol	—	−0.45	2,6-Dinitrophenol	−0.24	−0.34
o-nitrophenol	—	−0.40	3,4-Dinitrophenol	−0.24	−0.52
2,3-Dinitrophenol	−0.25	−0.40	2,4-Dinitro-*o*-cresol	−0.24	−0.47
2,4-Dinitrophenol	−0.25	−0.48			

[a]Potentials were measured versus Ag/AgCl in equal volumes of methanol and 0.20 M acetate buffer. Final pH was 5.4.

generally acceptable in the pH range 3—6 and in the methanol range 40—55%.

Initial experiments were done with a mobile phase consisting of equal volumes of methanol and 0.20 M acetate buffer of pH 4.5. The pH value measured in the mixed mobile phase was 5.4. Under these conditions, the sequence of elution was 2,6-, 2,4-, and 2,3-dinitrophenol followed by the overlapping peaks for *p*-nitrophenol, *m*-nitrophenol, 2,5-dinitrophenol, and 3,4-dinitrophenol. As expected from its internal hydrogen bonding, *o*-nitrophenol was the last to elute. The elution sequence for the dinitrophenols corresponds to the decreasing acid dissociation constants. The same relationship holds between *p*-nitrophenol and *m*-nitrophenol.

Varying the percent methanol expands or contracts the chromatograms along the time axis. Increasing the percent methanol decreases capacity factors (k'), whereas decreasing the percent methanol increases capacity factors, but the decreased peak heights lower sensitivity. For our locally packed columns, 45% methanol was judged to be optimum, although, with more efficient columns, a higher percentage of methanol in the mobile phase might be preferable.

Selectivity factors among the mononitrophenols are independent of methanol concentration from 40 to 55% and pH from 3.25 to 6.00. This effect seems reasonable because the thermodynamic pK values range from 7.15 to 8.4, and therefore no significant change in ionization would occur in these experiments. However, the thermodynamic pK values for the dinitrophenols range from 3.71 to 5.42, and these compounds showed large changes in selectivity factors as the mobile-phase pH was changed. Table 2 summarizes these observations; the selectivity factors given were calculated by dividing k' for each compound by k' for *p*-nitrophenol. The results of Mourey and Siggia [8] are consistent with an extension of Table 2 to the more acidic conditions that they used. However, the elution sequence reported by Roseboom et al. [9] with somewhat different conditions are not consistent with our results.

TABLE 2

Selectivity factors for nitrophenols (see text)

pH	CH_3OH (%)	*m*-	*o*-	2,6-	2,4-	2,3-	2,5-	3,4-
3.25	45	1.12	1.74	0.72	1.17	1.21	1.44	1.56
3.70	45	1.12	1.68	0.58	1.05	1.18	1.40	1.52
4.20	45	1.14	1.75	0.39	0.74	1.19	1.38	1.54
4.72	45	1.14	1.77	0.30	0.51	1.10	1.28	1.47
5.12	40	1.14	1.74	0.30	0.43	1.02	1.16	1.42
5.20	40	1.15	1.73	0.31	0.40	1.01	1.09	1.40
5.22	45	1.15	1.76	0.29	0.37	0.92	1.06	1.25
5.30	45	1.13	1.75	0.28	0.37	0.85	1.03	1.18
5.45	50	1.13	1.73	0.27	0.34	0.76	0.98	1.05
5.50	45	1.13	1.72	0.27	0.33	0.73	0.92	1.08
5.55	55	1.10	1.74	0.26	0.32	0.68	0.93	0.93
6.00	45	1.17	1.75	0.29	0.31	0.55	0.69	0.72
pK_a [7]		8.40	7.23	3.71	4.11	—	5.22	5.42

From the above information, a pH for the mobile phase can be selected that minimizes overlap between the chromatographic peaks. Among the mononitrophenols, no two compounds have overlapping chromatographic peaks. Among the dinitrophenols, the greatest overlap is between 2,5- and 3,4-dinitrophenol, but this overlap presents no problem for the electrochemical detector because the first reduction peaks of these two compounds occur at different potentials. The real problem develops when the chromatographic peaks for *p*-nitrophenol or *m*-nitrophenol overlap with a dinitrophenol. Then the dinitrophenol can be determined from its first reduction peak, but the mononitrophenol cannot be determined without complex data analysis.

Table 1 indicates that minimal interference on the potential axis occurs between *p*-nitrophenol and the second peak of 2,3-dinitrophenol. Therefore the pH of the mobile phase was selected so that these two compounds would coelute. A final pH of 4.9 was selected. Under these conditions, some overlap still occurs between *m*-nitrophenol and 2,5-dinitrophenol and between *p*-nitrophenol and 2,4-dinitro-*o*-cresol. However, each of these peaks can be seen in Fig. 2.

Figure 3 shows the output of the swept-potential electrochemical detector. The figure contains data without baseline subtraction,which can easily be done via the the computer system. The detector was operated with a 30-Hz square wave and the potential was swept from −0.18 to −0.66 V vs. Ag/AgCl in steps of −10 mV. The pulse size was −50 mV. Voltammograms were recorded every 2.5 s. The PARC 310 was used with large-sized drops which were replaced before each voltammogram. Figure 2 was obtained by summing all current values from the start of each voltammetric sweep to the

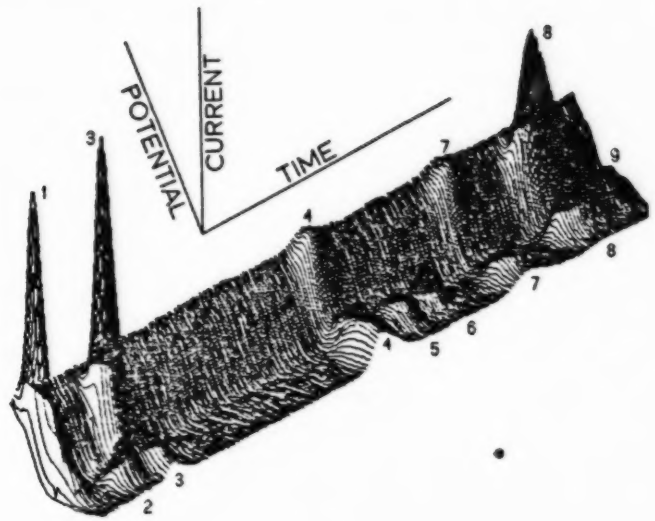


Fig. 3. 3-D chromatogram. Data are from the same experiment as Fig. 2. The time axis shows retention time from 4.4 to 15.6 min. See text for other conditions.

stated potential. It should approximate the results obtained with a d.c. electrochemical detector held at the stated potential and should also be similar to the result with a u.v. detector, although the relative peak heights would be different.

Figure 4 contains four chromatograms taken from the data file used for Figs. 2 and 3. Any one of the chromatograms of Fig. 4 consists of the current data for the same potential step of each voltammogram. Any potential, within the range swept, could have been used, and the four selected are only examples to point out salient features. Figure 4A is the chromatogram for −0.25 V and shows peaks for five dinitrophenols with no interference. The only dinitrophenol not included in this chromatogram is 2,5-dinitrophenol which has reduction peaks on each side of the potential selected here (see Fig. 1). At a less negative potential, 2,5-dinitrophenol is the only nitrophenol in the chromatogram. Figure 4B is the chromatogram for −0.39 V and shows a well-developed peak for orthonitrophenol. In addition, peaks for *m*-nitrophenol and the second peak for 2,5-dinitrophenol are evident. Figure 4C is for −0.48 V. Here, the peak for *o*-nitrophenol is only a shoulder on the peak for 2,4-dinitro-*o*-cresol, as it is in Fig. 2. The peaks for *m*-nitrophenol and 2,5-dinitrophenol are now well developed. In addition, a peak for an unknown impurity appears between those for 2,6- and 2,4-dinitrophenol. This peak can also be seen in Figs. 2 and 3. This unknown peak would not have been detected by a differential-pulse detector unless the potential was

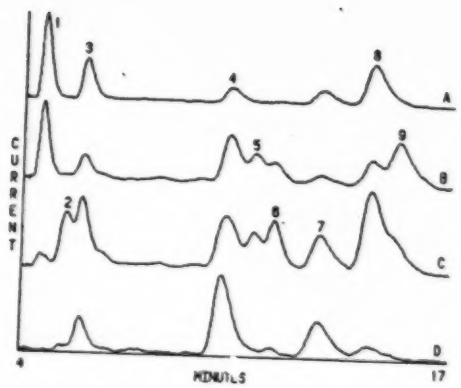


Fig. 4. Chromatograms at constant base potentials. Data are from the same experiment as Figs. 2 and 3. See text for explanation of A—D.

set near −0.48 V. A d.c. amperometric detector would not have detected the unknown unless its potential was more negative than approximately −0.45 V.

Figure 4D is for −0.61 V. The important peak here is for *p*-nitrophenol. Careful examination of Fig. 4 shows that this peak has shifted throughout the series 4A to 4D. In Fig. 4A, the peak represented pure 2,3-dinitrophenol. In the intermediate chromatograms, the peak represents a mixture of *p*-nitrophenol and 2,3-dinitrophenol. The compound nature of the peak can be seen in Fig. 3. Thus, the attempt to adjust the pH so that these two compounds would coelute was close but not perfect. Under these conditions, the *p*-nitrophenol elutes approximately 12 s before 2,3-dinitrophenol, consistent with the selectivity factors of Table 1.

It is obvious that the resolution between some of the peaks is not adequate for precise, straightforward quantitation. Several solutions are possible. In addition to using a column with greater resolution, it is possible to scale and subtract one curve from another. This process can be done on either a chromatogram as in Fig. 4 or a voltammogram as in Fig. 1. A future paper will deal with the mathematical deconvolution of such peaks. If the problem is simply to quantify one specific compound, the selectivity factors in Table 2 can be used to select a different pH for another experimental run. For example, *p*-nitrophenol can be separated from 2,3-dinitrophenol by changing the mobile phase to pH 6 or 3.25.

Square-wave voltammetry and the closely related technique of differential-pulse voltammetry are known to give linear response to changes in concentration over several orders of magnitude. Thus, the primary limit to linearity might come from chromatographic factors rather than from the detector. Although no careful study was done over an extended range of amount injected, peak height was linear with concentration until peak broadening became evident.

The sensitivity and limit of detection depend on several factors. Because the voltammetric peaks of the mononitrophenols are much lower than the first peaks of the dinitrophenols, the mononitrophenols have poorer sensitivities and limits of detection. Column resolution and retention time have obvious effects on peak heights. Flow rate is also important. The results of Samuelsson and Osteryoung [10] that the sensitivity increases with flow rate in this flow cell were confirmed. Sensitivity also increases with increasing size of the mercury drop. From the data for Fig. 3, the limit of detection for 2,6-dinitrophenol can be estimated to be slightly below 1 ng. An earlier experiment, with a smaller amount injected and different instrument settings, indicated a limit of detection of approximately 0.25 ng. Thus, the limits of detection are not as favorable as with a d.c. amperometric detector.

While the application of the swept-potential electrochemical detector discussed here is for h.p.l.c. in the reductive mode, the detector should be well suited to the oxidative mode with a wall-jet detector cell [11]. It might be necessary to include a pulse, before each voltammetric sweep, to some extreme potential to remove any reaction products from the electrode surface as suggested by Fleet and Little [11]. The detector should also find application to flow-injection systems and ion chromatography.

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